The Early Development of the summer egg of a Cladoceran (Simocephalus vetulus).

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With Plate 25 and 1 Text-figure.

Contents.

1. Introduction.

This paper deals chiefly with the method of germ-layer formation in the parthenogenetically-produced summer eggs of Simocephalus vetulus. A considerable amount of work has been done on the development of the eggs of various Cladocera, and a complete summary of this work is to be found in Vellmer's paper (**14**) on the development of winter eggs of Cladocera. In the same year that this paper appeared Kühn (**8**) described very fully the development of the summer egg of Polyphemus pediculus, and here again is given a résumé of the work that has been done on Cladoceran development. This paper also reviews, in this connexion,

all the work done on determinate development in the Crustacea. Since then no further work has appeared on this subject, so that it would be mere repetition if that work were to be again summarized here.

Vollmer (**14**), in his summary, states [1] 'that these results point to the fact that we must differentiate between two categories of eggs which possess different modes of development in relation to their yolk-content; eggs poor in yolk show a determinate development with practically total segmentation, eggs rich in yolk show an indeterminate superficial type of development'. Referring to his own work, he suggests that the developmental processes that he describes demonstrate an intermediate form between these two methods and make the transition less abrupt. From the work recorded in this paper it would seem that the development of the summer eggs of *S. vetulus* shows, perhaps more markedly, an intermediate stage between the determinate and indeterminate methods of development of Cladoceran eggs.

This work was carried out in Professor MacBride's laboratory at the Imperial College of Science, and I must thank Professor MacBride for valuable suggestions and for kindly reading the manuscript.

2. Method.

In all cases the embryos were dissected out of the brood-pouch into the smallest amount of water possible before being fixed. Fixing the whole Daphnid with the embryos still in the brood-pouch gave unsatisfactory results.

It was found necessary to employ different fixatives for the various stages of development. For the early stages no reliable method was found. Carnoy's fluid (Ac. Alc. Chloroform) gave good results, but the difficulty experienced was the unreliability of the fixative. The egg is surrounded by a tough membrane and it is this that causes the trouble. It is never possible to say whether it will burst or not under the action of the fixing agent. In the segmenting egg, if the membrane

[1] My translation.

bursts, the fixation is not good, while after the blastula stage the reverse is the case. If the membrane bursts, it produces a certain amount of distortion, and with all fixatives except Carnoy this was so bad as to make the material useless. With Carnoy a variable amount of swelling was produced, but with the other fixatives the whole egg usually burst. If the membrane remains intact, the egg becomes very difficult to embed owing to the embedding material not penetrating the membrane, and so it was found extremely difficult to obtain sections of the segmenting egg. One method used for the earliest stages was to employ hot water as the fixative. The eggs were dissected out of the brood-pouch and flooded with boiling water. After thirty seconds they were transferred to 70 per cent. alcohol. This gave fairly good results, but with later stages the nuclei were not well preserved and so the method was not of much use. Fixing in bichromate-formol and subsequent treatment with 5 per cent. formalin gave good results, but here again it was unreliable and gave results no better than those obtained with Carnoy's fluid. Gilson's mixture (Subl. Ac. Alc. Chloroform) gave very good fixation when it succeeded in fixing the embryo without producing excessive distortion.

For later stages Carnoy was again used, but better results were obtained with hot Flemming. The embryos were placed in Flemming's strong solution at 56° C. for ten minutes and then washed out in water. Strong picro-sulphuric gave fair fixation.

The embryos were stained with alcoholic eosin before clearing in clove oil and embedding in clove-oil 'celloidine'. This made them more conspicuous and hence easier to manipulate. After hardening the celloidine they were embedded in paraffin at 56° C.

Sections were cut 6 μ and 7 μ thick and stained on the slide. The best stain was Ehrlich's haematoxylin. Iron haematoxylin was used after Flemming fixation. Haemalum, picro-indigo-carmine and thionin were among other stains used which proved satisfactory.

3. Egg-laying.

On several occasions the actual laying of the egg into the brood-pouch was observed. Each egg is laid separately as a continuous stream of foam. The foam appears to consist of more or less opaque drops—probably yolk-spheres and transparent colourless globules—presumably oil in a continuous mass of protoplasm. Immediately after laying, the egg is of an irregularly elongated shape tapering at the end nearest to the opening of the oviduct. In a few minutes it has rounded itself off and become regularly shaped and almost spherical. The oil-drops now commence to coalesce to form one large oil-globule. About two hours after laying this large oil-drop is most distinct. It is excentrically placed in the egg and at this time has a diameter very slightly greater than half that of the egg.

As stated above, the only fixative that was found satisfactory for the earliest stages of the egg was Carnoy's fluid. In sections of eggs fixed in this liquid it was possible to recognize, according to Gatenby's diagnosis (**5**), the following structures: (1) one large oil-globule excentrically placed and surrounded by a few much smaller globules—these appeared as sections of empty vacuoles; (2) a mass of protoplasm placed almost centrally and on the edge of the large oil-globule; (3) a large number of yolk-discs staining very faintly with thionin and pervading the remainder of the egg; (4) a less number of smaller bodies scattered among the yolk-discs and staining deeply with thionin—presumably the remains of mitochondria or Golgi bodies.

Lebedinski (**9**) describes a similar arrangement of materials in the egg of *Daphnia similis*, but does not mention the mitochondria.

An egg-membrane is clearly distinguishable soon after the egg has been laid, and it would appear very probable, from the fact that the egg is laid as a fluid mass which subsequently rounds itself off, that this egg-membrane is produced by the egg itself after this rounding-off has taken place. It is not

a vitelline membrane if this term is restricted, as McMurrich (**10**) maintains, to a membrane which is connected with the process of fertilization, but must be termed a primary egg-membrane or 'Dotterhaut' as defined by Korschelt and Heider (**7**).

4. Cleavage.

Cleavage is completely superficial. At first the separate blastomeres remain deep in the egg as apparently amoeboid masses of protoplasm. After five hours they begin to appear on the surface, and soon after, each blastomere becomes separated from its neighbours by furrows extending a short distance into the yolk. Eight hours after the egg has been laid cleavage is complete and results in a uniform blastoderm enclosing the yolk-mass. No yolk-cells were found in the interior of the blastula. In Daphnia similis Lebedinski (**9**) found that certain blastomeres remained behind in the centre of the egg while the remainder migrated towards its surface to form the blastoderm, and that the former blastomeres functioned in absorbing the fat or yolk-drops. Vollmer (**14**) in the winter eggs of Cladocera describes the formation of a blastula with greatly reduced blastocoele by total cleavage, and states that cells are budded off from the blastomeres into the interior of the egg which function as yolk-cells. In Leptodora hyalina Samter (**12**) found that yolk-cells were budded off from the blastoderm at the same time that the endoderm plate commenced to immigrate into the egg. Agar (**1**) in Holopedium gibberum states that 'fairly late stages show occasional very flat nuclei on the separate yolk-masses, as figured by Samassa (**11**). Doubtless each yolk-mass is contained in a single cell. The origin of these yolk-cells has not been observed, but it may be safely assumed that they arise in the same way as that described by Samassa, i.e. by budding off from the mesendoderm'. Similarly, in S. vetulus embryos in which the endoderm has already separated from the mesoderm often contain yolk-masses against which flattened cells are seen to be lying. Their origin

cannot be stated with certainty, but it is thought very probable that they arise from cells originally lying round the genital rudiment which pass inwards on the inside of the blastoderm, as will be described below.

5. Formation of the Germ-layers.

The first sign of differentiation of the blastoderm is the appearance of a group of cells—more vacuolated than the rest—on one side of the embryo which subsequently proves to be the ventral side. These cells contain a large amount of yolk, and in their earliest stages their nuclei are very obscure. They will be called collectively the 'Ventral Mass' (fig. 1).

When cleavage is complete each blastomere consists of an inner yolky part and an outer non-yolky part. In their very earliest stages the cells of the ventral mass are completely pervaded by yolk and so are conspicuous by not showing the outer non-yolky zone. Soon a few of these cells pass inwards, so that the ventral mass becomes a small heap of vacuolated yolky cells on one side of the embryo, but as yet shows no further sign of differentiation.

The cells of the ventral mass on one side, which is seen later to be the anterior side, now proliferate and form a mass of yolky cells whose protoplasm stains comparatively deeply (fig. 2). The compactness of these cells and the distinct manner in which they are marked off from each other indicate that their protoplasm has a greater surface-tension than that of the cells of the remainder of the ventral mass. The nuclei of these cells, which are now becoming distinct, are large compared with those of the blastoderm cells—approximately twice as large. Their nucleoli are distinct and stain deeply.

Behind these cells, that is at the posterior part of the ventral mass, are a few cells which still form a single layer. They are very much vacuolated and contain a large amount of yolk. Their protoplasm does not stain at all deeply and the cells are not at all compact. At first their nuclei are not distinct, as with the remainder of the cells of the ventral mass, but soon these become quite clear and show very marked characteristics.

They are several times as large as those of the blastoderm cells, as will be seen from fig. 3. The chromatin in them is either very scattered or very scanty. Each nucleus contains several nucleoli which stain to varying degrees, but none stain at all deeply. These cells are the primordium of the gonads.

Commencing at the earliest stages when the nuclei of the cells of the ventral mass are still obscure, cells can be seen round the posterior periphery which are passing inwards and dorsally up the inside of the blastoderm. These cells are apparently formed by proliferation of the blastoderm cells round the edges of the ventral mass and then migrate inwards at its periphery. They are mesoderm cells and will be spoken of as the Ectomesoderm. Later their nuclei become more distinct and are seen to be larger than those of the blastoderm and to contain distinct deeply staining nucleoli.

Soon after the genital rudiment becomes distinct there appear on the dorsal side of the embryo the primordia of the nervous system—the 'Scheitelplatten'. These consist of two groups of tall columnar cells symmetrically placed about the median plane, in which the nuclei are large and oval, approximately twice as long as the nuclei of the neighbouring blastoderm cells. The nucleoli are deeply staining and very conspicuous, and there is a marked absence of chromatin in the remainder of the nucleus. They agree with those described by other workers on Cladocera, and their further development will not be treated here.

A very conspicuous change is now brought about in the embryo by the invagination of the genital rudiment. An early indication of this inward migration can be seen in fig. 3, where the surrounding cells are seen to be pushing their way over the primordial germ-cells. The primitive germ-cells sink into the egg, a variable but sometimes considerable distance. The pit caused by this sinking in has been seen to stretch a third of the way across the embryo. The lips of this pit are formed of the ectomesodermal cells which are continually pushing their way under the edge of the genital rudiment to lie on the inside of the blastoderm (fig. 4), and as the

invagination proceeds these lips gradually approach one another (figs. 7 and 8) and thereby tend to enclose a space which sometimes persists for a short time as a small cavity (fig. 5). The lips ultimately fuse (fig. 6), so that the primordium of the gonads comes to lie completely internally. With the closure of this invagination the passage of the ectomesodermal cells into the interior stops in this region.

At the time of invagination the number of cells constituting the genital rudiment is about ten, but there seems to be no constant number. Cell divisions among these cells were found but rarely. Vollmer (**14**) states: 'Teilungsfiguren habe ich aber niemals in der Gonadenanlage nachweisen können', but in S. vetulus the number of cells in the genital rudiment most certainly increases by cell division from about four at its earliest apparent differentiation to about ten at its invagination.

While these changes have been taking place at the posterior end of the ventral mass the formation of the 'mesendoderm' has commenced at the anterior end. The original compact yolky cells at this end apparently separate into two parts—an inner mass of cells which spread themselves as mesodermal cells over the anterior part of the blastoderm conspicuously in the region of the 'Scheitelplatten', and an outer region which remains as part of the blastoderm. In the centre of this region, that is about midway between the genital rudiment and the level of the 'Scheitelplatten', the mesendoderm makes its first appearance as a group of tall, compact, comparatively non-yolky cells in the blastoderm (fig. 7). The nuclei of these mesendoderm cells show at first no difference from those of the blastoderm cells, but later, as the mesendoderm mass grows, the nuclei are seen to be nearly double as large as the blastoderm nuclei, with conspicuous nucleoli. This enlargement can be seen to take place as the cells pass inwards. The mass enlarges and its posterior end pushes its way backward in the median plane (fig. 8). The area of origin, which may be termed the blastozone, is marked a little later by a depression from which later grows the stomodaeum.

In its backward growth the mesendoderm comes up against the primordium of the gonads. There is no strict relation between the times of mesendoderm formation and of the invagination of the genital rudiment—sometimes the latter is completely internal before the mesendoderm begins to grow posteriorly. The mesendoderm pushes its way underneath the genital rudiment between this and the blastoderm, which may now be called ectoderm, so that the genital rudiment comes to lie on the dorsal side of the mesendoderm (fig. 9).

During the formation of the mesendoderm, mesoderm cells are formed at the periphery of the blastozone, most conspicuously at the anterior and lateral borders, the posterior border being obscured by the backwardly-growing mesendoderm. The mesoderm at this stage is grouped, in the posterior portion of the embryo, ventro-laterally, while in the anterior part it extends dorsally, covering the 'Scheitelplatten'.

When the mesendoderm has finished its backward growth it is a very clearly defined mass, and is sharply separated from the lateral mesoderm, as can be seen in fig. 11. It now begins to flatten out, and its lateral borders cease to be sharply cut off from the neighbouring mesoderm. Ultimately the whole of the mesoderm and mesendoderm form one flat plate of cells lining the inside of the ventral ectoderm. While this fusion is taking place the nuclei of the mesoderm and mesendoderm cells become smaller, so as to be indistinguishable from those of the ectoderm. From this plate of cells in the median plane a solid rod of cells separates off, which is the endoderm (fig. 10). At this stage the rudiments of the second antennae are already showing. Much later, when the large stomodaeum and the smaller proctodaeum have grown in from the ectoderm, this solid rod acquires a lumen.

At the time of separation of the endoderm the genital rudiment still exists in the ventral part of the embryo lying on the gut, as a mass of yolky cells with very large nuclei showing the same characteristics as the original primordial germ-cells of the ventral mass.

6. Discussion.

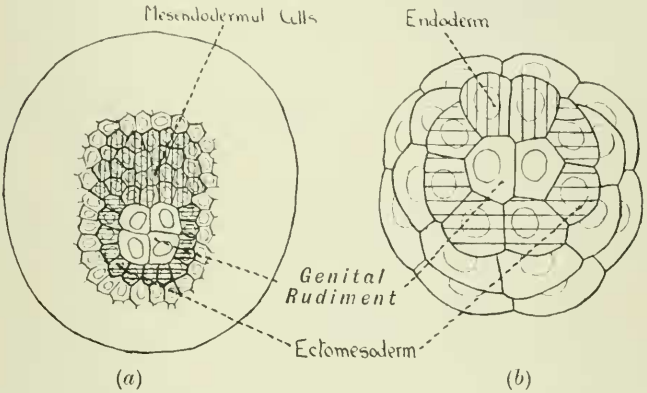
Kühn has shown in his paper dealing with the development of the summer eggs of Polyphemus (**8**) that the early development of Moina as described by Grobben (**6**) is very similar, in fact almost identical, to that of Polyphemus, and Samassa's description (**11**) of a totally indeterminate method of development of the eggs of Moina receives no support from his work. These are the only two Cladocera in which a determinate type of development has as yet been described.

Vollmer in his work on the resting eggs of the Cladocera (**14**) states that when the blastoderm consists of about two hundred cells a migration inwards takes place of about eight to ten of these from the future ventral side of the embryo. These multiply and form the genital rudiment. A similar proliferation of cells from a ventral blastozone later forms the 'untere Blatt', and from this is subsequently separated a solid rod of cells which forms the gut. Because of the early separation of the genital rudiment Vollmer states that this method of development is intermediate between the determinate development of Polyphemus and Moina and the indeterminate type of development as described by Agar (**1**) in Holopedium and Lebedinski (**9**) in Daphnia.

A comparison of the genital rudiment as described by Vollmer for Daphnia with that of Simocephalus described in this paper shows certain differences. Firstly, the mode by which it passes into the interior of the embryo is different in the two cases. In Daphnia this is brought about by a few cells that wander from the blastoderm into the interior of the egg, presumably by the action of an inwardly directed cytotaxis. In Simocephalus, on the other hand, the group of cells forming the genital rudiment passes into the interior by an invagination and only becomes internal when the edges of the pit caused by this invagination have grown together and fused. Here again the invagination may be brought about by a similar force. However, the extent of the invagination varies considerably, sometimes the pit is very

shallow, while at other times, as stated above, it has been seen to stretch one-third of the way across the egg. This fact, when it is also remembered that the surrounding cells are actively proliferating and producing cells which push their way inwards between the edge of the genital rudiment and the blastoderm, suggests that the invagination may be brought about by the ectomesoderm cells pushing the genital rudiment

TEXT-FIG. 1.



(*a*) Diagram of the ventral view of embryo of Simocephalus vetulus, showing the ventral mass before the formation of the mesendoderm.
(*b*) Ventral view of embryo of Polyphemus pediculus in thirty-two-cell stage (from Kühn).

in front of them as they themselves pass into the embryo. A second difference lies in the fact that in Daphnia the primordial germ-cells when they have passed into the interior lose their yolk. Vollmer states (**14**): 'auch in den Blastodermzellen schreitet die Dotterresorption fort, wenn auch nicht in demselben Grade wie in der Gonadenanlage'. In S. vetulus the cells of the genital rudiment always consist of large yolky cells which retain their yolk all through the development. Their protoplasm also stains very faintly, not as in Daphnia, where Vollmer states that these cells show an increased affinity for stains. However, from the position of origin of the

genital rudiment in the two forms, and from its relation to the mesendoderm and ultimate fate, it would seem that the differences are of small significance and that the two structures described as genital rudiment are really homologous.

A comparison of the mode of development of S. vetulus with that of Polyphemus as described by Kühn reveals some very close analogies. Text-fig. 1 (*a*) shows a diagram of the ventral mass of S. vetulus before the formation of the mesendoderm. In the posterior region are the large primordial germ-cells bordered laterally and posteriorly by ectomesodermal cells. In front is the group of yolky cells which are mesendodermal. The inner layers of this latter cell-mass spread out over the anterior part of the blastoderm as mesodermal cells, and from the outer layer is developed the very definite mesendoderm. While this is growing backwards mesoderm cells are still being proliferated inwards at the anterior and lateral edges of this group and possibly at the posterior edge. The fact that these latter cells originate by proliferation of cells at the edge of this mesendodermal group, together with the fact that they form mesoderm distinct from the mesoderm included in the backwardly growing mesendoderm, suggests that possibly they are a separate source of mesoderm, that they are ectomesodermal cells—a continuation forwards of the ectomesodermal cells which are formed at the periphery of the genital rudiment. If this were so, an analogy might be drawn with the development of Cyclops as described by Urbanowicz (**13**), where he states that larval mesenchyme arises from cells surrounding the primitive endoderm cell while the secondary mesoderm arises from the gut. The more recent work of Fuchs (**4**) on Cyclops has, however, failed to confirm the findings of Urbanowicz, and has, on the contrary, demonstrated an extraordinary resemblance between the development of Cyclops on the one hand and Polyphemus and Moina on the other, in neither of which is there any larval mesenchyme as distinct from secondary mesoderm. But in S. vetulus when the mesendoderm is growing backwards, although its hinder end is very sharply separated

from the laterally lying mesoderm (fig. 11), at the anterior end no such clearness exists and at the blastozone the mesendoderm merges into the plate of mesoderm lining the anterior part of the embryo. But both this anterior mesoderm and the mesendoderm clearly arise from a sharply defined group of cells at the blastozone, and it is suggested that there is no distinction between the mesoderm of the mesendoderm and the other mesoderm formed in this anterior region. If this is so, a very complete analogy can be found with Polyphemus. Text-fig. 1 (*b*) shows a view of the vegetative pole of a Polyphemus embryo in the thirty-two-cell stage. Two central primordial germ-cells forming the genital rudiment are placed posteriorly to two cells which give rise to the whole of the endoderm. Laterally and posteriorly to the genital rudiment are six cells which give rise to both ectoderm and mesoderm. Each of these six cells divides into two cells, one of which becomes an ectoderm cell and the other gives rise to mesoderm cells. In the comparison of these two figures it is seen in the two cases that the germ-cells are completely segregated in the genital rudiment as two cells in Polyphemus and as a group of about four cells in S. vetulus. Forming a crescent posteriorly round this primordium in both cases are mesectodermal cells, but anteriorly in Polyphemus are two endoderm cells, while in S. vetulus are a group of mesendoderm cells. The chief difference between the two forms is thus that the endoderm is segregated very late in S. vetulus, while it separates very early in Polyphemus—in the sixteen-cell stage. Similarly the mesoderm is segregated later than the endoderm, but still very early in Polyphemus compared with S. vetulus where the separation of mesoderm is only complete with the separation of the endoderm.

In Moina and Polyphemus Weismann (**15**) has proved that the parents nourish the young in their brood-pouch, and it is probably due to this fact that the yolk in the eggs of these two forms has diminished so considerably, and in correlation with this disappearance of yolk is the appearance of the teloblastic type of development. In S. vetulus

and also in Daphnia Agar (**2**) has shown that while the embryo is in the brood-pouch it does not receive nourishment from its parent. And yet S. vetulus shows a type of development which differs considerably from that of Daphnia in that there is a very early segregation of the genital rudiment but shows such obvious similarities to the development of Moina and Polyphemus.

The fact that has been pointed out by Fuchs (**4**), that among groups so far apart as the Copepoda and the Cladocera, in forms where there has been loss of yolk owing to the development of other modes of nutrition of the embryo, there is such an extraordinary similarity in the cell lineages, suggests firstly that the arrangement of the 'Anlagen' in the eggs of these forms is a very archaic character, and secondly that in cell lineage there is a representation of the arrangement of the 'Anlagen' in the yolky eggs that do not show a teloblastic mode of development. This view is upheld by the similarity between the cell lineages of the Cladoceran eggs that contain little yolk and of the egg of the Cirripede Lepas, where although there is abundant yolk, yet there is determinate cleavage (Bigelow, **3**). In the development of S. vetulus there is further support of this view in that in this apparently indeterminate method of development the earliest arrangement of the germ-layer 'Anlagen' shows such a close resemblance to the arrangement of the teloblasts in the non-yolky eggs of the Cladocera.

7. Summary.

1. Each egg is laid as a yolky mass of a foam and later forms a primary egg-membrane.

2. Cleavage is completely superficial and apparently indeterminate.

3. The first differentiation of the blastoderm is the appearance of a group of vacuolated yolky cells on the ventral side of the embryo which are called the ventral mass.

4. This subsequently differentiates into a few large cells with very large nuclei which form the genital rudiment, surrounded laterally and posteriorly by ectomesodermal cells,

and anteriorly to this a mesendodermal mass of cells from which arises the mesendoderm.

5. The genital rudiment surrounded laterally and posteriorly by inwardly growing ectomesodermal cells invaginates and becomes internal by the lips of the invagination growing together and fusing.

6. The mesendoderm grows backwards as a solid mass of cells, which later spreads out flat and becomes indistinguishable from the laterally-lying mesoderm, and from this layer the endoderm separates as a solid rod in the median plane.

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8. Bibliography.

1. Agar, W. E. (1908).—"Note on the early development of a Cladoceran (Holopedium gibberum)", 'Zool. Anz.', 33.
2. —— (1913).—"Transmission of environmental effects from parent to offspring in S. vetulus", 'Phil. Trans. Roy. Soc. Lond.', 203.
3. Bigelow, M. A. (1902).—"The early development of Lepas", 'Bull. Mus. Harvard Coll.', 40.
4. Fuchs, K. (1913).—"Die Zellfolge der Copepoden", 'Zool. Anz.', 42.
5. Gatenby, J. B. (1919).—"The Identification of Intracellular Structures", 'Journ. Roy. Micro. Soc.'
6. Grobben, C. (1879).—"Die Entwicklungsgeschichte der Moina rectirostris", 'Arb. a. d. Zool. Inst. Wien', 2.
7. Korschelt and Heider (1903).—'Lehrbuch der vergleichenden Entwicklungsgeschichte der wirbellosen Thiere.'
8. Kühn, A. (1913).—"Die Sonderung der Keimbezirke in der Entwicklung der Sommereier von Polyphemus pediculus (de Geer)", 'Zool. Jahrb., Abt. f. Anat.', 35.
9. Lebedinski, J. (1891).—"Die Entwicklung der Daphnia aus dem Sommerei", 'Zool. Anz.', 14.
10. McMurrich, J. P. (1895).—"Embryology of the Isopod Crustacea", 'Journ. Morph.', 11.
11. Samassa, P. (1893).—"Die Keimblätterbildung bei den Cladoceren: I. Moina rectirostris (Baird)", 'Arch. Mikr. Anat.', 41.
12. Samter, M. (1900).—"Studien zur Entwicklungsgeschichte der Leptodora hyalina", 'Zeit. f. wiss. Zool.', 68.
13. Urbanowicz, F. (1884).—"Zur Entwicklungsgeschichte der Cyclopiden", 'Zool. Anz.', 7.
14. Vollmer, C. (1912).—"Zur Entwicklung der Cladoceren aus dem Dauerei", 'Zeit. f. wiss. Zool.', 102.
15. Weismann, A. (1877).—"Beiträge zur Naturgeschichte der Daphnoiden", ibid., 28.

EXPLANATION OF PLATE 25.

List of Abbreviations.

bl, blastozone; *ect*, ectoderm; *em*, ectomesoderm; *end*, endoderm; *ga*, genital rudiment; *gac*, cavity of genital rudiment; *i*, pit produced by invagination of genital rudiment; *me*, mesendoderm; *mes*, mesoderm; *mm*, mesendodermal mass; *v.m*, ventral mass; *y.c*, yolk-cells.

Figs. 1, 2, 3, 4, 8, and 9, are from material fixed in Carnoy's fluid. The remainder are from Gilson material.

Fig. 1.—Section through an embryo showing the earliest sign of differentiation of the blastoderm. The ventral mass is marked off from the rest of the blastoderm as a group of cells completely pervaded by yolk.

Fig. 2.—Median section through embryo showing differentiation of ventral mass into (1) genital rudiment, (2) anteriorly, the comparatively deeply staining mesendodermal mass, and (3) posteriorly, the ectomesoderm cells which are passing inwards. The nuclei at this stage are not at all distinct.

Fig. 3.—Slightly oblique section—almost median—of an embryo slightly older than that figured in fig. 2. Shows the same as in fig. 2, but nuclei are now distinct. The cells surrounding the genital rudiment are seen to be pushing their way over the latter.

Fig. 4.—Transverse section of the genital rudiment showing how the lips of the pit caused by its invagination are formed of inwardly migrating ectomesoderm cells.

Fig. 5.—Transverse section through the genital rudiment after it has become completely internal, showing its cavity.

Fig. 6.—Transverse section through the invaginating genital rudiment showing the fusion of the lips of the invagination pit.

Fig. 7.—Median section showing commencement of mesendoderm. The genital rudiment is not yet completely internal.

Fig. 8.—Median section showing mesendoderm growing backwards from the blastozone which is marked by a small depression.

Fig. 9.—Median section. The mesendoderm has grown backwards underneath the genital rudiment which is now completely internal.

Fig. 10.—Transverse section showing endoderm separated as a solid rod from the laterally lying mesoderm. The genital rudiment is immediately dorsal to the endoderm. Yolk-cells are seen in this figure enclosing yolk and oil-drops.

Fig. 11.—Transverse section through the posterior region of the blastozone showing mesoderm formation at the lateral borders of the blastozone.